HAYATI Journal of Biosciences, December 2009, p 151-156 Vol. 16, No. 4
ISSN: 1978-3019

Fatty Acid Synthesis by Indonesian Marine Diatom, *Chaetoceros gracilis*

ALBERTA RIKA PRATIWI[1], DAHRUL SYAH[2], LINAWATI HARDJITO[3], LILY MARIA GORETTI PANGGABEAN[4], MAGGY THENAWIDJAJA SUHARTONO[2]*

[1]Department of Food Technology, Soegijapranata Catholic University, Jalan Pawiyatan Luhur IV/1, Bendan Duwur, Semarang 50234, Indonesia
[2]Department of Food Science and Technology, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[3]Aquaculture Product of Technology, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[4]Research Centre for Oceanography, Indonesian Institute of Science, Jalan Pasir Putih No. 1 Ancol, Jakarta 14430, Indonesia

Received October 8, 2009/Accepted December 28, 2009

Since the primary storage nutrients in diatoms consist of lipid, they are potential for the industrial fatty acid production. High value fatty acids include arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. This study aimed to analyze fatty acid synthesis by *Chaetoceros gracilis* diatom during growth. There was a large increase in lipid yield from 4pg cell^{-1} mass of lipid per cell at the exponential phase to 283pg cell^{-1} at stationary phase. The lipid concentrations also increased significantly from the stationary phase to the death phase, but not significantly from the end exponential phase to the stationary phase. The relative percentage of saturated fatty acid (SAFA) of the total fatty acid was higher than that of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) at all of growth phase. The highest PUFA was found at stationary phase at the same time when SAFA was being the lowest. The majority of SAFA was palmitic acid (24.03-40.35%). MUFA contained significant proportion of oleic acid (19.6-20.9%). Oleic acid, linoleic acid and á-linolenic acid were found at every stage growth. These fatty acids are considered as precursor for production of long chain PUFA-Docosahexaenoic acid (DHA/22:6ù3) through series of desaturation and elongation step with all of desaturase enzyme (Ä8-D, Ä9-D, Ä12-D, Ä15-D, Ä17-D, Ä6-D, Ä5-D, and Ä4-D) and elongase enzyme (E).

Key words: *Chaetoceros gracilis*, fatty acid, synthesis, saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA)

INTRODUCTION

PUFA have been recognized as having a number of important neutraceutical and pharmaceutical applications. Data on the fatty acid distributions of a large number of microalgae species including diatom have been reported. Since it is known that the major food storage of diatom is lipid, there had been many exploration of diatom as one of the potential sources of fatty acids, in particular PUFA (Lebeau & Robert 2003).

A number of environmental or culturing factors influence the fatty acid composition of diatom. The growth phase in batch culture system is very important factor in the formation of lipid and fatty acid. The nutrient deficiency affects synthesis activity of the lipid enzyme. There is currently a resurgence of interest in the fatty acid composition and associated metabolism of marine diatom. Yap and Chen (2001) reported that oleaginous microorganisms such as diatom tend to store their energy source in the form of lipids as the culture age. That is indicate that growth phase in batch culture is an important factor, which can influence the lipid content and fatty acid compositions.

Biosynthesis of polyunsaturated fatty acid comprises of two processes. One is the de novo synthesis of saturated or monounsaturated fatty acid from acetate and the other is the conversion of these fatty acids to polyunsaturated fatty acid through a series of desaturation and elongation processes (Yap & Chen 2001). *Phaeodactylum tricornutum* had eight routes for EPA formation, i.e. four routes from 18:2 (n-6) to 20:5 (n-3); two routes pass through (n-3)-fatty acids and one route through (n-6)-fatty acid as intermediates. The other route passes through both (n-3)- and (n-6)-fatty acid as intermediate (Arao & Yamada 1994). However, little is known about fatty acid synthesis in other diatom.

Chaetoceros gracilis is one of the marine diatoms, which is easily cultured, with the characteristic of high growth rate. This diatom is also specific and abundant in Indonesia. There had been many studies on this diatom such as lipid content and fatty acid compositions but no report on the fatty acid composition during growth. Discussion on the possible enzymes involved in this synthesis presented in this manuscript.

MATERIALS AND METHODS

Culture Condition. The axenic culture of *C. gracilis* diatom was provided by Mariculture Laboratory of Research Centre for Oceanography–Indonesian Institute of Science (LIPI). The diatom was cultured in natural enriched f/2-silicate Guilard medium. The medium contains mayor nutrient (0.99 mM $NaNO_3$, 0.07 mM $NaH_2PO_4 \cdot 2H_2O$, 5.28 µM $Na_2SiO_3 \cdot 9H_2O$); minor nutrient (5.36 µM $FeCl_3 \cdot 6H_2O$ and 26.86 µM Na_2EDTA),

*Corresponding author. Phone: +62-251-8330559, Fax: +62-251-621724, E-mail: mthenawidjaja@yahoo.com

vitamins (0.59 µM vitamin B1, 0.001 µM vitamin B12, 0.004 µM biotin) and trace metal (0.781 µM $CuSO_4 \cdot 5H_2O$, 2.12 µM $ZnSO_4 \cdot 7H_2O$, 0.521 µM $NaMoO_4 \cdot 2H_2O$, 0.005 µM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 18.19 µM $MnCl_2 \cdot 4H_2O$, 0.61 µM $CoCl_2 \cdot 6H_2O$). The medium was adjusted to pH 8 and 28 ‰ of salinity. The batch culture was maintained at 16-19 °C, 12 h light/12 h dark periodic at 4000-5000 Lx using fluorescent tubes as the light source and aerated continuously.

Cell density was monitored every day by counting cell with a Neubauer haemocytometer chamber. The cells were harvested from the end exponential until death phase by centrifugation at 5000xg for 15 min 4 °C.

Extraction, Saponification and Esterification of Lipid. Diatom cells were sonicaticated for 3 x 3 sec at 20 KHz at 16 micron amplitude (Soniprep 150 MSE) in 5 ml $CHCl_3$-MeOH-H_2O (5:10:4) solution. The combined extract was reacted with $CHCl_3$:H_2O (1:1) solution to give a final solution ratio of $CHCl_3$-MeOH-H_2O (10:10:9). Hereinafter, lipid was recovered in chloroform phase by removing solvent under N_2 gas. Weighing at this step gives the total of lipid content (Dunstan *et al*. 1994).

The total lipid extract was saponified by 100 ml of 0.5 M KOH/MeOH solution to form free fatty acid. The free fatty acid were esterified to form Fatty acid methyl esther (FAME). It was esterified with 175 ml of 20% of BF3/MeOH solution. The solution was boiled for 2 min and mixed with a small volume of concentrated isooctane then boiled again for 2 min. Following this step, 15 ml of saturated sodium chloride (20%) was added to the mixture at room temperature and shacked strongly until two phases were formed. The upper phase (isooctane and lipid phase) was dissolved with 25 ml of petroleum benzene (40-60 °C) and filtered with sodium thiosulfat present on filter paper, the filtrate was evaporated with N_2 gas. After esterification step, fatty acid methyl ester (FAME) was redissolved in 1 ml of *n*-hexane and an aliquot of 1ul was used for chromatography gas analysis.

Fatty Acid Analysis. FAME were identified by GC/MS. The aliquot of 1 ml samples was injected on CG/MS QP-5000 with a DB-17 column (30 m long and 0.25 mm i.d). Temperature of both injector and detector were 250 °C. After 1 min, the temperature was raised 100 °C for 3 min and continuously 10 °C min^{-1} until 230 °C for 3 min then further to 260 °C. This final temperature was maintained for 10 min. The Pressure of gas was 64.5 Kpa with the flow rate being 1.0 ml/min.

The analysis of fatty acid synthesis is studied based on fatty acid composition of each stage of growth from end-exponential phase to death phase.

RESULTS

Growth and Lipid Production. The culture conditions of *C. gracilis* were controlled under the conditions known to produce healthy cell. The change of lipid content was studied on several stages of growth phases: end-exponential phase (stages 1), early-stationary phase (stage II), stationary phase (stages III), end of stationary phase (stage IV), and death phase (stage V). There was a large increase in lipid yield (mass of lipid per cell) from 4pg $cell^{-1}$ at stage I (3d) to 233pg $cell^{-1}$ at stage II (7d) (ca.58x) (Figure 1). The concentrations of lipid also increased from stage II (7d) (233pg $cell^{-1}$) to stage V (17d) (721pg $cell^{-1}$) (ca.3x), but not as drastically as from stage I to stage II.

Saturated and Unsaturated Fatty Acid. The fatty acid pattern of the *C. gracilis* can be divided based on its saturation, namely saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). During growth, the SAFA content decreased (29.53%) from end-exponential until stationary phase and increased (47.38%) again at death phase, whereas MUFA declined continuously from exponential phase to the death phase. PUFA concentration was related inversely to SAFA content, when the SAFA was decreased, PUFA was found increased (Figure 2). The PUFA was increased from end-exponential to stationary phase then descended through the death phase.

During growth, composition of SAFA, MUFA, and PUFA showed different at each stage of growth. Especially of PUFA of *C. gracilis*, its compositions were more unsaturated at death phase.

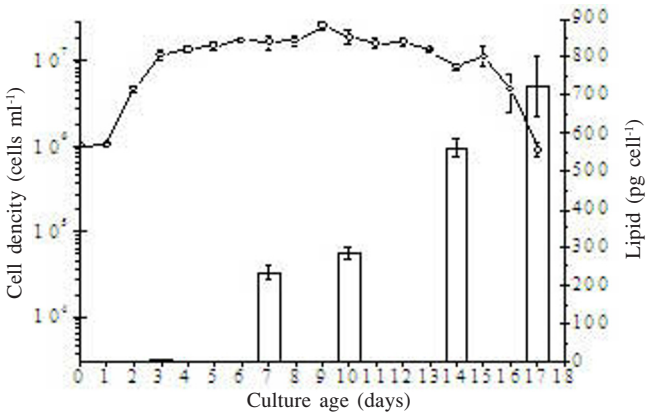

Figure 1. Growth curve and lipid concentration during growth of *C. gracilis*. Lipid was analyzed at day 3, 7, 10, 14, and 17. ○: growth curve, □: lipid concentration. Bar indicate standard errors n = 2.

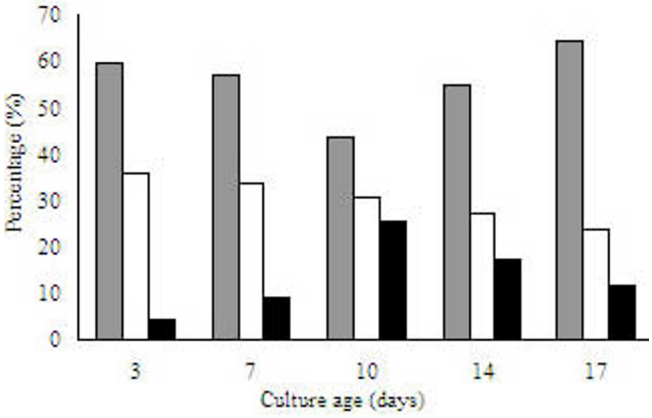

Figure 2. Relative proportion (% of total fatty acid) of saturated fatty acid (SAFA), monoun saturated fatty acid and polyunsaturated fatty acid (PUFA) of *C. gracilis* diatom during growth. ■: SAFA, □: MUFA, ■: PUFA.

Fatty Acid Synthesis During Growth. SAFA was the dominant fatty acid in all stages, but the fatty acid compositions were different in all growth phase. The primary component of SAFA was palmitic acid that was also found in all growth phase. Other saturated fatty acid usually found in other diatom was also found in this study, for example myristic acid and lauric acid. The last two fatty acis were formed in all growth stages whereas stearic acid was initially formed in the stationary phase until the death phase, while pentadecanoic acid (15:0) and arachidic acid (20:0) were not formed in diatom even in a very small amount (Table 1).

The most important MUFA found in this diatom was oleic acid, which was seen in all growth stages with concentrations of 17-21% of the total fatty acid. The other MUFA was palmitoleic acid, which was found only at the end-exponential phase and cis-vacenat was only found in the early-stationary phase. The tetradecanoic acid, petroselinic acid and erucic acid were found in very small number and only during the stationary stage. PUFA found in *C. gracilis* diatom showed diverse degree of saturation at different growth phases.

The finding of PUFA with lower saturation degrees such as linoleic acid, γ-linolenic acid, α-linolenic acid, eicosadienoic acid, eicosatrienoic acid, dihomo-γ linolenic acid in each growth stage is related with biosynthesis of long chain PUFA, which are known to have high economic value such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). During growth, AA was only found during stationary until death phase and DHA was only found in death phase, whereas the EPA was not found in any growth phase (Table 1).

DISCUSSION

Growth and Lipid Production. The increased of lipid content during growth from the exponential phase until the death phase might be the result of accumulation of total lipid associated with the nutrition deficiency. Generally, this result was similar to that of other diatom reported by previous researchers. Pernet *et al*. (2003) reported that the lipid total of *C. mueleri* diatom increased was related to silicon-depletion. Whereas, lipid accumulation of *Chaetoceros* cf. *wighamii* diatom is usually was triggered by nutrient deficiency (Araùjo *et al*. 2005).

Saturated and Unsaturated Fatty Acid. During growth, SAFA was the dominant fatty acid compared to MUFA and PUFA. This is similar to result found in *Thalassiosira pseudonana* diatom, whereas the SAFA of *P. tricornutum* diatom were lower than MUFA (Tonon *et al*. 2002). The composition of SAFA, MUFA, and PUFA in microalgae can be changed due to differences in culture media, environmental conditions also culture age (Yap & Chen 2001; Mansour *et al*. 2003; Rousch *et al*. 2003). Lower environmental temperature induced increase in unsaturated fatty acid synthesis. This is a response to maintain the cell membrane fluidity. At lower temperature, the fatty acid of the cell membrane will be more unsaturated and more stable. *C. gracilis* did not synthesis PUFA completely when cultured at temperature of 25-28 °C (data is not shown). Nevertheless, diatom *C. gracilis,* which was cultured at temperature of 16-19 °C synthesized PUFA, although saturated fatty acid stays higher than unsaturated fatty acid (MUFA and PUFA).

Table 1. Fatty acid relative proportion (% of total fatty acid) of *C. gracilis* diatom during growth phase

Fatty acid		Fatty acid percentage of total fatty acid				
		3 d	7 d	10 d	14 d	17 d
Saturated fatty acid (SAFA)						
12:0	Lauric acid	6.97	0.53	0.94	0.73	0.42
14:0	Myristic acid	15.86	20.32	13.01	18.78	19.44
15:0	Pentadecanoic acid	0.98	1.21	0.87	1.29	1.75
16:0	Palmitic acid	35.75	32.83	24.03	31.50	40.35
18:0	Stearic acid	-	2.18	3.23	1.83	2.12
20:0	Arachidic acid	-	-	1.53	0.62	0.20
% total SAFA		59.57	57.08	43.61	54.76	64.28
Monounsaturated fatty acid (MUFA)						
14:1Ä13	Tetradecynoic acid	-	0.99	0.71	-	-
16:1Ä9	Palmitoleic acid	14.44	-	-	-	-
18:1Ä6	Petroselinic acid	0.67	-	0.79	0.61	2.21
18:1Ä9	Oleic acid	20.94	31.05	17.42	23.04	19.66
18:1Ä11	vaccenic acid	-	1.79	7.53	3.85	2.34
22:1Ä1	Erucic acid	-	-	4.37	-	-
% total MUFA		36.05	33.83	30.82	27.49	24.21
Polyunsaturated fatty acid (PUFA)						
16:2Ä7,10	Hexadecadienoic acid	0.51	-	0.86	0.88	1.03
18:2Ä5,8	Octadecadienoic acid	0.68	-	-	-	-
18:2Ä9,12 (ù 6)	Linoleic acid	3.19	1.18	0.59	1.14	1.19
18:3Ä6,9,12 (ù 6)	ã-linolenic acid	-	-	-	0.35	0.44
18:3Ä9,12,15 (ù 3)	á-linolenic acid	-	2.24	1.26	2.33	2.15
20:2Ä11,14 (ù 6)	Eicosadienoic acid	-	1.91	21.46	9.97	-
20:3Ä11,14,17 (ù 3)	Eicosatrienoic acid	-	3.12	0.96	0.46	0.75
20:3Ä8,11,14 (ù 6)	Dihomo-ã linolenic acid	-	0.64	-	-	-
20:4Ä5,8,11,14 (ù6)	Arachidonic acid (AA)	-	-	0.43	1.05	1.39
20:5Ä5,8,11,14,17 (ù3)	Eicosapentaenoic acid (EPA)	-	-	-	-	-
22:6Ä4,7,10,13,16,19 (ù3)	Docosahexaenoic acid (DHA)	-	-	-	-	4.56
% total PUFA		4.38	9.09	25.56	17.75	11.51

Differences of SAFA, MUFA, and PUFA content were also influenced by culture age. In case of *C. gracilis* diatom, PUFA content increased until stationary phase and then gradually decreased at death phase. Many others diatom sometimes have PUFA increased significantly as the culture older, because it related to energy storage (Yap & Chen 2001).

Fatty Acid Biosynthesis During Growth. Palmitic acid (16:0) was the primary of SAFA found in this study (Table 1). These fatty acids are assumed as characteristic of *Bacillariophyceae*, which is usually high in its palmitic acid content (Mansour *et al*. 2003). Formation of palmitic acid is related to the energy storage requirenment. Therefore, palmitic acid is always found in all of growth stages. Tonon *et al*. (2002) observed high increase in palmitic acid at another diatom *P. tricornutum* and *T. pseudonana* during the later culture stage. This is related to the extra energy required by cells division.

Biosynthesis of long chain PUFA is started from oleic acid (18:1Ä9) (Yap & Chen 2001). Decreasing of oleic acid consentration is in line with the increasing of PUFA (Figure 3), implying that the oleic acid might act as the substrate for formation of the long chain fatty acid. PUFA formation involves series of process of desaturation and elongation catalyzed by desaturase and elongase enzymes (Yap & Chen 2001).

The oleic acid was dominant in diatom at all growth phases, followed by linoleic acid. This might imply of Ä12 desaturase enzyme acting on oleic acid substrate to produce linoleic acid (18:2Ä9,12) (Yap & Chen 2001). The discovery of linoleic acid at all growth phases showed that in *C. gracilis* diatom production of long chain PUFA potentially is available at all times. Linoleic acid (LA) is the parent of omega 6 (ω6) fatty acid synthesis and also act as substrate for production of α- linolenic acid/ ALA (18:3Ä9,12,15-ω3) which in turn become parent of the omega 3 (ω3) fatty acid synthesis (Figure 6). Formation of α-linolenic acid during early stationary to death phase implied that Ä15 desaturase enzyme (Wen & Chen 2003) might be actively used linoleic acid as substrate in the process of desaturation. Linoleic acid is decreased because it was also used as the substrate to produce other fatty acid in the metabolic pathway such as eicosadienoic acid (20:2Ä11,14-ω6) and γ-linolenic acid. Both γ-linolenic acid (18:3Ä6,9,12-ω6) and eicosadienoic acid (20:2Ä11,14-ω6) were found at early-stationary until end-stationary phase and the death phase respectively (Table 1). Therefore, Ä6-desaturase and Ä9 elongase enzyme might work on linoleic acid substrate during this stage (Figure 6). Delta 5 desaturase enzyme (Ä6 desaturase) also catalyzed conversion of α-linolenic acid (18:3Ä9,12,15-ω3) substrate to octadecatetraenoic acid (18:4Ä6,9,12,15-ω3) (Yap & Chen 2001), but in this study, fatty acid (eicosatrienoic acid/ 20:3Ä11,14,17-ω3) was formed (Table 1). Therefore, Ä9-elongase enzyme might be also at work (Figure 6), namely catalyzed conversion of 18:3 to 20:3 (Domergue *et al*. 2002; Meyer *et al*. 2004).

The delta 6 elongase enzyme (Ä9 elongase) used α-linolenic acid (18:3Ä9,12,15-ω3) substrate to produce dihomo-γ-linolenic acid (20:3Ä8,11,14-ω6) (Figure 6), which was formed during stationary phase in *C. gracilis*, even though at very small percentage (0.64% total of fatty acid) (Table 1). According to Khozin *et al*. (1997), dihomo-γ-linolenic acid (20:3Ä8,11,14(n-6) could be formed using two substrates namely γ-linoleic acid (18:3Ä6,9,12-ω6) and eicosadienoic acid (20:2Ä11,14-ω6) with elongase enzyme and Ä8 desaturase enzyme catalysis (Figure 4). In this study, *C. graclis* also produced eicosadienoic acid (Table 1).

Despite dihomo-γ-linolenic acid being found at small percentage and was not formed until the cell reached death phase, but arachidonic acid/AA (20:4Ä5,8,11,14-ω6) was found during these phases which further increased until death phase (Table 1). Therefore, dihomo-γ-linolenic acid (20:3Ä8,11,14-ω6) could possibly the substrate to form AA (Figure 6) through desaturation reaction. According to Wen and Chen (2003), the desaturase 5 enzyme could use dihomo-γ-linolenic acid substrate in the production of AA (20:4-ω6).

Biosynthesis of long-chain PUFA (EPA and DHA) could be started from AA (20:4-ω6) and/or docosatetraenoic acid (20:4-ω3) substrates (Yap & Chen 2001; Domergue *et al*. 2002; Wen & Chen 2003). Unlike other diatoms, *C. gracilis* did not synthesize 20:4-ω3 and EPA, but directly produced DHA at high percentage (4.56% of total fatty acid/ 39.61% of total PUFA) (Table 1). The DHA concentration of other species

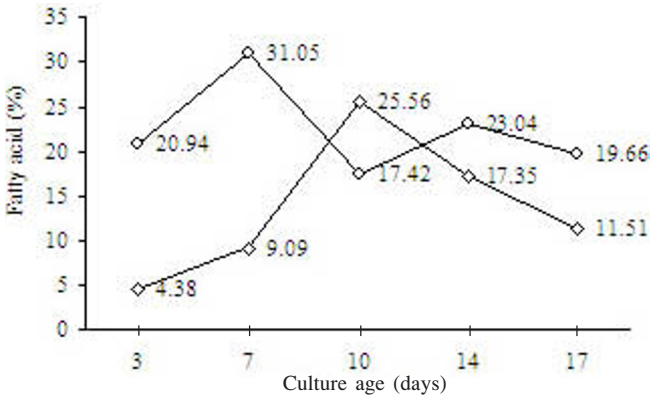

Figure 3. Oleic acid concentration (proportion relative of total fatty acid) as substrate to polyunsaturated fatty acid (PUFA) production during growth. ◇: PUFA, ○: Oleic acid.

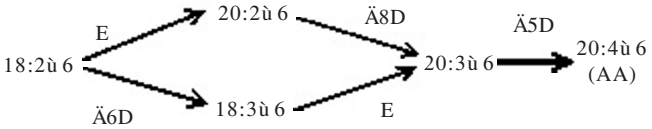

Figure 4. Arachidonic acid/AA (20:4-ù 6) formation through eicosadienoic acid (20:2ù 6) and ã-linolenic acid (18:3ù 6) which is catalized by Ä8 desaturase (Ä8D), Ä5 desaturase (Ä5D) and elongase (E) enzyme (Khozin *et al*. 1997). D: desaturase enzyme, E: Elongase enzyme.

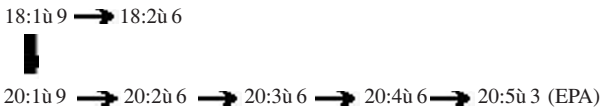

Figure 5. One of models of eicosapentaenoic acid (EPA) production with oleic acid (18:1ù9) as a precursor through desaturation and elongation step (Domergue *et al*. 2002).

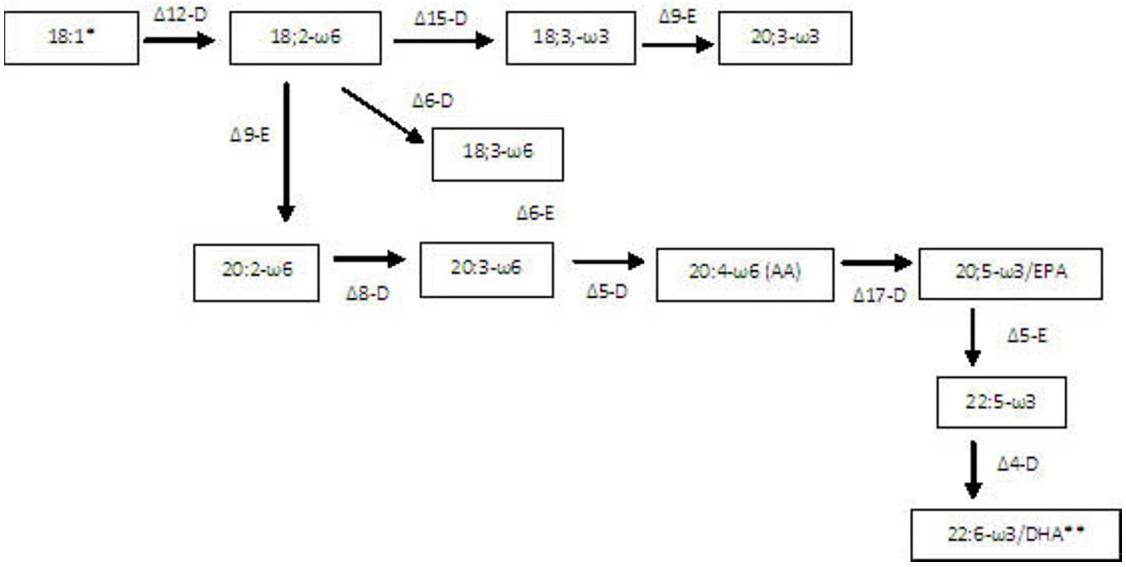

Figure 6. Proposed biosynthesis pathway of DHA in *C. gracilis* diatom. *: high percentage of total fatty acid and, **: high percentage of total PUFA. (Ä12-D) : delta 12 desaturase enzyme, (Ä15-D): delta 15 desaturase enzyme, (Ä17-D): delta 17 desaturase enzyme, (Ä6-D): delta 6 desaturase enzyme, (Ä8-D): delta 8 desaturase enzyme, (Ä5-D): delta 5 desaturase enzyme, (Ä4-D): delta 4 desaturase enzyme, (Ä9-E): delta 9 elongase enzyme, (Ä6-E): delta 6 elongase enzyme, (Ä5-E): delta 5 elongase enzyme.

Chaetoceros sp., *C. affinis, C. calcitran* was found at 0.8%, 0.1 and 1.2% of their total fatty acid respectively (Servel *et al.* 1994; Viso & Martin 1993; Renaud *et al.* 1999). While EPA was not detected (Table 1), this might imply that EPA act as the substrate to form DHA in metabolic pathway.

Therefore, EPA, and DHA might be synthesized through AA pathway (Figure 6). Wen and Chan (2003) reported that this phenomenon was caused by the possibility of the activity of Ä17 desaturase enzyme, which used AA to form EPA. The enzyme inserted double bond into the end methyl of hydrocarbons chain. Furthermore, EPA was used as substrate until being diminished to produce DHA. Delta 5 elongase (Ä5 elonage) and Ä4 desaturase enzyme might also work on EPA substrate to DHA formation (Meyer *et al.* 2004) (Figure 6).

When viewed from fatty acid biosynthesis pathway above, DHA formation in this diatom, follow route of biosynthesis that was based on the route of omega 6 biosynthesis pathway using eicosadienoic acid 20:2Ä11,14-ω6 substrate to form AA, EPA, and DHA. This pathway can be a combination pathway between AA formation route (Khozin *et al.* 1997) (Figure 4) and one of scheme route of EPA biosynthesis (Figure 5) as reported in *P. tricornutum* diatom (Arao & Yamada 1994; Domergue *et al.* 2002).

ACKNOWLEDGEMENT

This research was supported in part by Hibah Bersaing-DIKTI-2007/2008. No.151/SP2H/PP/DP2M/III/2007.

REFERENCES

Arao T, Yamada M. 1994. Biosynthesis of polyunsaturated fatty acid in the marine diatom, *Phaeodactylum tricornutum*. *Phytochemistry* 35:1177-1181.

Araùjo Sirlei de Castro, Maria V, Garcia T. 2005. Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* brightwell under different temparature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. *Aquaculture* 246:405-412.

Domergue F, Lerchl J, Ulrich Za¨ hringer, Heinz1 E. 2002. Cloning and functional characterization of *Phaeodactylum tricornutum* front-end desaturases involved in eicosapentaenoic acid biosynthesis. *Eur J Biochem* 269:4105-4113.

Dunstan GA, Volkman JK, Barret SM, Leroi JM, Jeffrey SW. 1994. Essential polyunsaturated fatty acid from species of diatom (Bacillariophyceae). *Phytochemistry* 35:155-161.

Khozin I, Adlerstein D, Bigogno C, Heimer YM, Cohen Z. 1997. Elucidation of biosynthetis of eicosapentaenoic acid in the microalga *Porphyridium cruentum*, studies with radiolabeled precursors. *Plant Physiol* 114:223-230.

Lebeau T, Robert JM. 2003. Diatom cultivation and biotechnology relevant products: Part II. Current and putative products. *Appl Microbiol Biotechnol* 60:624-32.

Mansour MP, Volkman JK, Blackburn SI. 2003. The effect of growth phase on the lipid class, fatty acid and sterol composition in the marine dinoflagellate, *Gymnodinium* sp. In batch culture. *Phytochemistry* 63:145-153.

Meyer A *et al.* 2004. Novel fatty acid elongase and their use for the reconstruction of docosahexaenoic acd biosynthesis. *J Lipid Research* 5:1899-1909.

Pernet F, Tremblay R, Demers D, Roussy M. 2003. Variation of lipid class and fatty acid composition of *Chaetoceros muelleri* and *Isochrysis* sp. Grown in a semicontinuous system. *Aquaculture* 221:393-406.

Renaud SM, Thinh LV, Parry DL. 1999. The gross chemical composition and fatty acid composition of tropical Australia microalgae for possible use in mariculture. *Aquaculture* 170:147-159.

Rousch JM, Scott SE, Sommerfeld MR. 2003. Changes in fatty acid profiles of thermo-intolerant and thermo-tolerant marine diatoms during temperature stress. *J Exp Mar Biol Ecol* 295:145-156.

Servel MO, Claire C, Derrien A, Coiffard L, De Roeck-Holtzhauer Y. 1994. Fatty acid composition of some marine microalgae. *Phytochemistry* 36:691-693.

Tonon TD, Harvey, Larson TR, Graham IA. 2002. Long chain polyunsaturated fatty acid production to triacylglycerols in four microalgae. *Phytochemistry* 61:15-24.

Viso AC, Marty JC. 1993. Fatty acid from 28 marine microalgae. *Phytochemistry* 334:1521-1533.

Wen ZY, Chen F. 2003. Heterotrophic production of eicosapentaenoic acid by microalgae. *Biotec Adv* 21:273-294.

Yap CY, Chen F. 2001. Polyunsaturated Fatty Acids: Biological Significance, Biosynthesis, and Production by Microalgae and Microalgae-Like Organism. In: Chen F, Jiang Y (eds). *Algae and Their Biotechnology Potential*. Netherland: Kluwer Acad Publ.